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Seed germination of valuable high-altitude medicinal plants of southern Africa

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Germination of nine important medicinal plant species from the high altitudes of southern Africa was investigated in relation to different environmental parameters. The seeds were subjected to different constant and alternating temperatures, temperature shifts, light and dark conditions, and cold stratification periods. Temperature regimes of 17–23°C appear most suitable for optimal germination for all the species examined. In some species, a temperature shift from 10°C to 20°C and 30°C to 20°C improved the final percentage germination. In the majority of the species investigated, exposure of seeds to continuous or alternating light significantly promoted germination over continuous dark. However, no phytochrome effect was determined. *Tulbaghia alliacea* and *Dianthus basuticus* germinated equally well in light and dark, while *Urginea capitata* responded significantly to the continuous light treatment. In some species, cold stratification stimulated germination and reduced the mean germination time.

Introduction

The 20th century has led to an increased demand for plant medicines. Surveys have estimated that 20 000 tonnes of plant material are traded annually in South Africa (Mander 1997). Rural South Africans are dependent mainly upon indigenous medicinal plants for their primary health-care (Kendler *et al.* 1992, Cunningham 1994, Mander 1998). In Lesotho, several important medicinal plant species — all with restricted distribution ranges — used in traditional medicine are becoming vulnerable (Talukdar 2002). If the future demand for medicinal plants is to be met, it is imperative that many of the species utilised in traditional medicine be domesticated and commercially cultivated (Van Staden 1999, Jäger and Van Staden 2000). To meet this increasing demand it is desirable to standardise techniques for efficient cultivation practices. The successful cultivation of medicinal plants from southern Africa is determined to a large extent by germinability of the seeds. Unlike other cultivated crops there is little or no pertinent information about the environmental factors responsible for germination of these plants. Hence, comprehensive knowledge about the environmental conditions required for seed germination of medicinal plants is an important prerequisite for propagation strategies. In this study, nine high-altitude medicinal plants widely utilised by local inhabitants of South Africa and Lesotho were selected and their seed germination response to different environmental factors such as temperature, temperature shifts, dark and light conditions, and cold stratification evaluated.

The medicinal plants investigated in this study were *Anthospermum rigidum* Eckl. and Zeyh., *Dianthus basuticus* Burtt Davy, *Dichilus strictus* E. Mey., *Gladiolus dalenii* van Geel, *Kniphofia caulescens* Bak., *Rumex lanceolatus* Thunb., *Scabiosa columbaria* L., *Tulbaghia alliacea* L.f. and *Urginea capitata* (Hook.) Bak. The roots of *A. rigidum* are used to treat painful menstruation and are also used during pregnancy by the Sotho (Watt and Breyer-Brandwijk 1962). *Dianthus basuticus* is used as a medicine to increase the fertility of bulls (Guillarmod 1971). The plant *D. strictus* is burnt with *Buchenroedera viminea* and the ashes strewn on all inhabitants of a household to give protection from lightning (Guillarmod 1971). The corms and leaves of *G. dalenii* are burnt and the smoke inhaled to clear a stuffy nose (Roberts 1990). Guillarmod (1971) reported that decoctions of *K. caulescens* are used to cure pains in the shoulders. *Rumex lanceolatus* is taken as a traditional remedy for internal parasites (Watt and Breyer-Brandwijk 1962). Dried, roasted roots of *S. columbaria* are made into a wound-healing ointment, and the powdered roots are also used as a pleasant-smelling baby powder (Von Koenen 1996). *Tulbaghia alliacea* was an early Cape remedy for fever (Forbes 1986) and is also used as a purgative, for fits, rheumatism and paralysis (Watt and

Breyer-Brandwijk 1962, Hutchings *et al.* 1996). *Urginea capitata* is traditionally used as a powerful good luck charm (Pooley 1998).

Materials and Methods

Seed material

Seeds of the species mentioned earlier were collected from the high-altitude areas of Lesotho (2 290m to 3 480m asl) and its South African surroundings. Lesotho is a small country, lying between 28°35'/30°40' South and 27°00'/29°30' East. Seeds were collected between March and May 2002. Immediately after collection the seeds were stored for about a year in paper bags at room temperature (25 ± 0.5°C) before being used.

Viability and imbibition studies

Viability was determined using 2,3,5-triphenyl tetrazolium chloride (TTC) solution (International Seed Testing Association 1999). The seeds of all the plant species were imbibed for 24h in water and further soaked in 1% colourless solution of TTC for 16h at 25°C in the dark. Seeds with red-stained embryos were recorded as viable. In imbibition studies, the seeds were placed in Petri dishes on filter paper (Whatman No. 1) moistened with distilled water and allowed to imbibe at room temperature (25 ± 0.5°C). At intervals of 6h, 18h, 24h and 48h the seeds were blotted dry, weighed and returned to wet filter paper. The amount of water imbibed by seed is expressed as percentage increase in seed weight.

Germination studies

In all experiments, due to a relatively low number of wild seeds available, four replicates of five seeds (in some species 10 seeds) were germinated on two layers of Whatman No. 1 filter paper moistened with distilled water. Seeds were surface decontaminated with 0.1% mercuric chloride for 2min and then rinsed under distilled water prior to germination tests. Disposable plastic Petri dishes (9cm) were used. Germination was tested at a 16h-light and 8h-dark photoperiod with cool-white fluorescent lamps, which provided a photosynthetic photon flux density of 45.60 ± 4.84µmol m^{-2} s^{-1}. Germination was recorded daily and was considered complete once the radicle protruded to about 2mm in length. The experiments were terminated after 40 days. Mean germination time (MGT) was calculated by using the following equation: $MGT = \sum(n \times d) / N$, where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment (Ellis and Roberts 1981).

To determine the effect of different temperatures, the seeds of all species were incubated in growth chambers at 10°C, 20°C, 25°C, 30°C and 15/30°C. The optimum temperature for germination of the seeds of a specific species was calculated on the basis of constant temperature: $T_o = \sum tp / \sum p$, where p is the percentage germination at temperature t (Olff *et al.* 1994). In continuous dark treatment, the Petri dishes were placed in light-proof boxes and the seeds were inspected daily under green 'safe light' with a photosynthetic photon flux density of 0.3µmol m^{-2} s^{-1}. In continuous light treatment the photosynthetic photon flux density was maintained at 16.46µmol m^{-2} s^{-1}. In both treatments the seeds were germinated at room temperature of 25 ± 0.5°C.

For the evaluation of the effect of 15 and 30 days of cold stratification on seed germination, seeds were placed between two layers of paper towel moistened with distilled water inside plastic bags stored at 5°C. After the desired period of cold stratification, germination tests were performed as outlined above, at 25 ± 0.5°C. Seeds not subjected to cold stratification served as control.

Phytochrome studies

Seeds were decontaminated with 0.1% mercuric chloride for 2min, subsequently rinsed with distilled water and imbibed in the dark at 25°C for 10min before being exposed to the light treatments for 5min and 30min. The light treatments consisted of dark control, white light control, red light (8.46µmol m^{-2} s^{-1}), far-red light (1.36µmol m^{-2} s^{-1}) and alternating red to far-red light. All seeds were incubated in the dark at 25 ± 0.5°C for 14 days, except for the white light control. Germination was recorded under a green 'safe light'.

Statistical analysis

The germination data in each treatment were arcsine-transformed (Sokal and Rohlf 1995) and analysis of variance (ANOVA) was conducted on the transformed data. All data were analysed using GENSTAT® Release 4.21. The Least Significant Difference (LSD) at the 5% level was used to test differences between means of percentage germination (Steel and Torrie 1960). Chi-square analysis was used to compare the stratification treatment at 95% confidence value.

Results and Discussion

Seed viability

A quick and easy method of checking the viability of seeds is to use the tetrazolium test (Cottrell 1947). The viability as determined by the TTC test in seed-lots of *A. rigidum* and *S. columbaria* was 80% and 90% respectively. Other species exhibited ≥96% viability after storage of about a year.

Water uptake

Water absorption depends on the permeability of the seed coat (Perissé 1997), the internal seed characteristics (Bradford 1995) and, to some extent, the seed surface-area-to-volume ratio (Shephard and Naylor 1996). For this reason, it is necessary to determine the amount of water required for imbibition and the germination processes to take place in each of the species and varieties. In this study, an amplified pattern of water absorption was recorded in all the plant species. The highest percentage of fresh weight increase (349%) and hence water uptake was recorded for

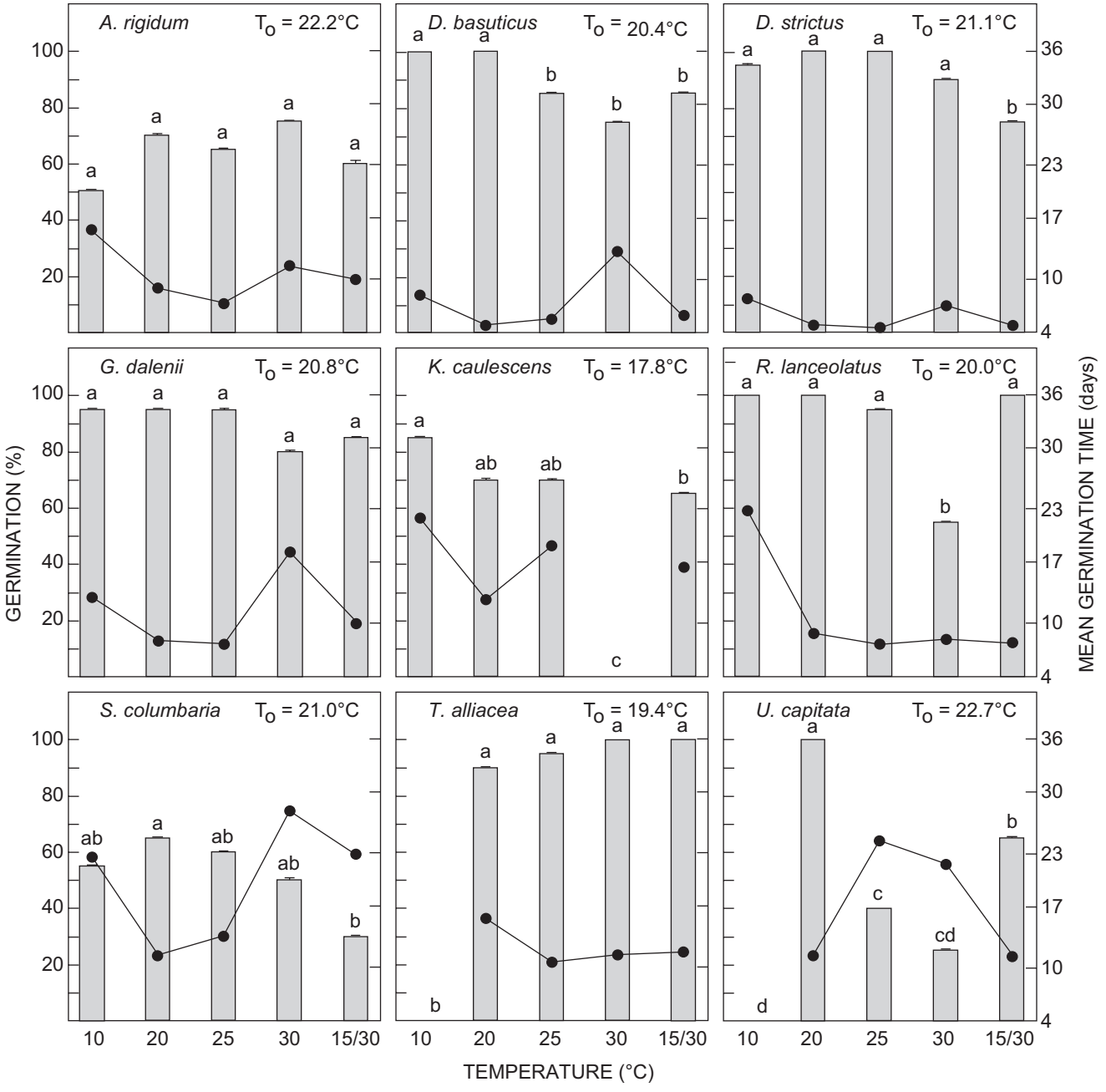

Figure 1: Effect of different temperatures on seed germination of high-altitude medicinal plant species from southern Africa. T_o = optimum temperature. Values within each species with the same letter are not significantly different ($P \leq 0.05$). Percentage germination is indicated by bars, and mean germination time is indicated by line points

D. strictus. None of the species examined exhibited any restrictions in water uptake, as there was a consistent increase in water uptake at hourly intervals at room temperature.

Effect of constant and alternating temperatures

Germination requirements are species specific (Datta 1965) and the extent and rate at which the germination process occurs in a non-dormant seed is affected by environmental factors such as temperature, light, oxygen, carbon dioxide and factors affecting the availability of water (Mayer and Poljakoff-Mayber 1975, Bewley and Black 1982, 1994). Several environmental factors simultaneously affect germination, but temperature is often regarded as the most important factor in determining the timing of germination (Badger and Ungar 1989). The effects of temperature on seed germination of the examined species are summarised in Figure 1. Results show that constant and alternating temperatures had a significant effect on seed germination of

some species. Seeds of *D. basuticus*, *K. caulescens* and *R. lanceolatus* showed lower germination percentages at a higher temperature (30°C). *Dianthus basuticus* had the highest germination at 10°C and 20°C with 100% germination at each temperature, and the lowest MGT was observed at 20°C. Lower temperature (10°C) inhibited germination of *T. alliacea* seeds. The germination of *U. capitata* seeds was significantly inhibited at low and high temperatures. In *D. strictus*, seeds germinated under alternating temperatures (15/30°C) had a significantly lower percentage germination compared to seeds germinated under constant temperatures. However, no significant differences in percentage germination were recorded between high and low constant temperatures. Constant and alternating temperatures had no significant effect on the percentage germination of *A. rigidum* and *G. dalenii* seeds. *Anthospermum rigidum* had 50% or more germination and *G. dalenii* more than 80% germination at all the tested temperatures. In the case of *G. dalenii*, the MGT was shortened as temperature increased. *Rumex lanceolatus* showed a sharp decrease in MGT with an increase in temperature. *Scabiosa columbaria* showed 65% germination at 20°C, which did not differ significantly with other temperatures, except with alternating temperatures with only 30% of germination. *Urginea capitata* seeds subjected to 20°C gave 100% germination, which declined significantly with other temperatures. At 10°C, germination was totally inhibited. Therefore, on the basis of the above findings, the optimum germination temperature requirements of all the species investigated ranged between 17°C and 23°C. In the natural habitat seeds are exposed to alternating and not constant temperatures (Baskin and Baskin 1998). However, constant temperatures appear to be most suitable for germination in *D. strictus*, *K. caulescens* and *S. columbaria*. Other species in this study performed equally well at both constant and alternating temperatures.

Effect of a temperature shift

Seeds germinate over a wide range of temperatures, but the maximum and minimum temperatures for germination vary with each species (Baskin and Baskin 1998). At a low temperature of 10°C, seed germination in *Erythrina burana* (Teketay 1994), *Prosopis farcta* (Dafni and Negbi 1978) and *Sesbania drummondi* (Eastin 1984) is inhibited. The seeds of *T. alliacea* did not germinate after 40 days of incubation at a low temperature (10°C), and were shifted to 20°C. The temperature shift exhibited stimulatory effects on seed germination of *T. alliacea*, giving 100% germination. Similarly, the seeds of *K. caulescens*, which did not germinate at 30°C, gave 35% germination when shifted to 20°C.

Effect of light conditions

Light sensitivity of seeds is suggested to have some relation to seed germination in their natural habitat (Mayer and Poljakoff-Mayber 1989). With regard to the light regime, percentage germination of *A. rigidum*, *D. strictus*, *K. caulescens*, *R. lanceolatus*, *S. columbaria* and *U. capitata* was significantly greater under constant light conditions than under constant darkness (Figure 2). In the majority of the species, exposure of seeds to continuous and alternating light conditions (16:8h light/dark) promoted germination, with shorter MGT than the dark conditions. *Dianthus basuticus* and *T. alliacea* germinated equally well under all conditions. As reported by Baskin and Baskin (1998), non-dormant seeds of many species germinate equally well in light and dark. *Gladiolus dalenii* seeds showed stimulatory effects under continuous dark periods relative to continuous light, achieving 95% germination. Continuous dark conditions had a negative impact on seeds of *K. caulescens*, *R. lanceolatus* and *S. columbaria*, resulting in no germination. According to Grime *et al.* (1981), seeds of most species have higher

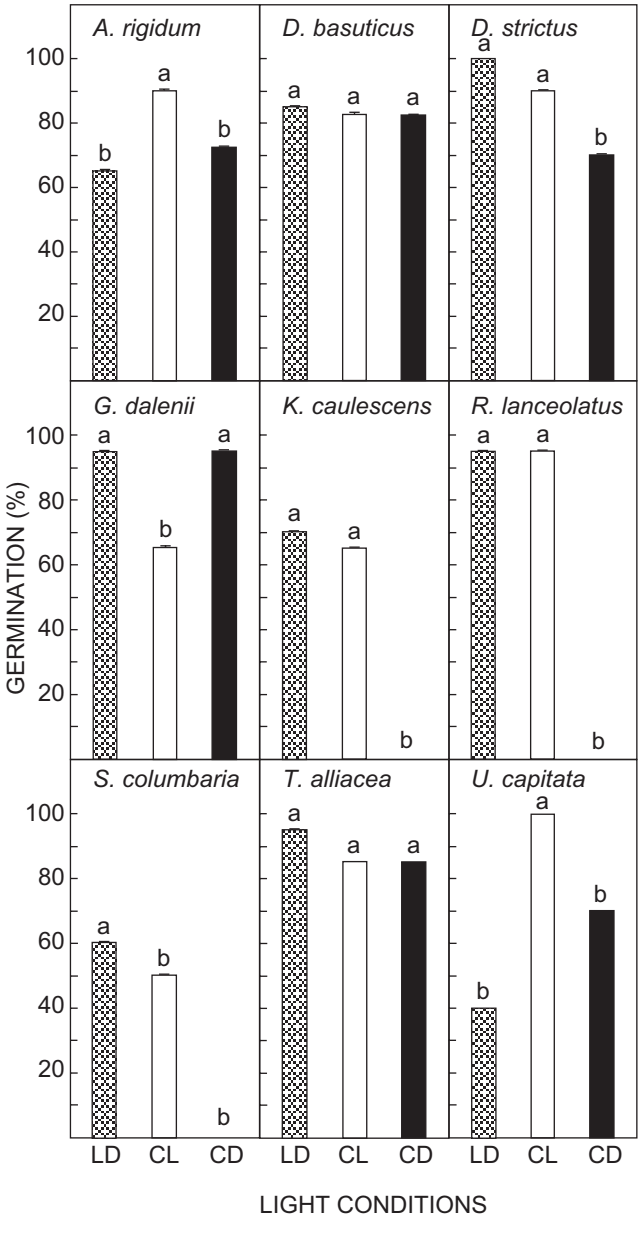

Figure 2: Effect of different light conditions on seed germination of high altitude medicinal plant species from southern Africa. LD = 16:8h light/dark, CL = continuous light, CD = continuous dark. Values within each species with the same letter are not significantly different ($P \leq 0.05$)

germination in light than in darkness. Relatively few species have seed that germinate better in darkness than in light (Baskin and Baskin 1998). These results show similar findings, with most of the species responding to light and few of them to the dark conditions. In general, the germination response of a seed to light also depends on the interaction with other environmental factors such as temperature, water potential and chemicals (Pons 1992, Bewley and Black 1994). The light-requiring species, when exposed to red and far-red light for a period of 5min and 30min, had no significant difference in percentage germination in comparison to the constant light control. These results suggest that there is no phytochrome effect present at short exposure periods. Possibly, with a longer or constant exposure to red and far-red light, effects of phytochrome may be detected.

Effect of cold stratification

Seeds of many species after-ripen during storage in moist conditions at low temperatures, a treatment known as 'stratification' (Mayer and Poljakoff-Mayber 1989). Both the temperature and length of stratification can be important factors for germination. In the present study, some species were sensitive and their seeds germinated during the stratification period. The seeds of *D. basuticus*, *G. dalenii* and *R. lanceolatus* had greater than 50% germination during the 15 days of stratification period. This is not surprising, as they grow at high altitudes where low temperatures and snowfalls occur for three to four months of the year. These were not considered for further experimental trials. *Urginea capitata* seeds exhibited a pronounced effect with a two-fold increase in percentage germination after 30 days of stratification, and it also halved the MGT in comparison to the non-stratified seeds (Table 1). Thirty days' cold stratification significantly improved the percentage germination of *A. rigidum*. Seeds of *K. caulescens* and *T. alliacea*, when exposed to various stratification periods, did not show any significant improvement in germination. The MGT, however, was reduced from 19 to 9 days in *K. caulescens* and from 11 to 8 days in *T. alliacea* after 30 days of stratification. Stratification of *D. strictus* seeds for 30 days achieved 100% germination, but it did not differ significantly from the control, and had a longer MGT. Similar results were obtained in *S. columbaria* seeds, with 15 days of stratification resulting in 80% germination and the MGT being reduced from 14 to 11 days after 30 days of stratification. Thullen and Eberts (1995) have shown that the seeds of *Scirpus acutus* had higher germination success when stratified for 8 or 12 weeks. Mirov (1936) observed that stratification treatments increased germination of seeds from plants growing at elevations greater than 1 200m. Baxter *et al.* (1993) also reported that exposure of high-altitude *Themeda triandra* seed to moist chilling progressively increased germination. Overall, from the present investigation, it was noted that seeds of *A. rigidum*, *D. basuticus*, *G. dalenii*, *R. lanceolatus* and *U. capitata* showed a positive response to cold stratification, while others did not have any significant response.

The objective of this study was to investigate the influence of environmental factors on seed germination of highland medicinal plants. After storing the seeds of all the species for a period of one year, 80–96% viability was maintained. This indicates their orthodox or intermediate storage characteristics. Suggesting that, it is possible to store the seeds at room temperature for up to a year and still retain viability. Water-uptake studies showed that none of the species had seeds with coat-imposed dormancy. The optimum temperature for germination of all the species ranged between 17–23°C. This implies that germination in the field will be largely restricted to the onset of summer, when the high altitudes of Lesotho and its South African surroundings experience temperatures of 20–25°C and high summer rainfall (85% between October and April). This study indicates that the sowing of seeds can be done just before the summer for successful seed germination. The germination at soil surface or at depths proximal to the soil surface indicates the role of light in seed germination. In the present study, the light was an important factor for *A. rigidum*, *D. strictus*, *K. caulescens*, *R. lanceolatus*, *S. columbaria* and *U. capitata*, which gave higher percentage germination in light than in dark. This suggests

Table 1: Effect of cold stratification on seed germination (%) of high-altitude medicinal plant species from southern Africa. Values in parentheses indicate mean germination time (MGT)

Plant species	Stratification period (days)		
	0	15	30
A. rigidum	65.0 ± 0.27 (7.18)	80.0 ± 0.00 (7.75)	85.0 ± 0.25* (7.48)
D. strictus	100 ± 0.00 (4.40)	65.0 ± 0.23** (10.8)	100 ± 0.00 (8.56)
K. caulescens	70.0 ± 0.47 (18.9)	60.0 ± 0.25 (11.4)	65.0 ± 0.49 (8.34)
S. columbaria	60.0 ± 0.25 (13.6)	80.0 ± 0.25 (13.4)	55.0 ± 0.25 (10.5)
T. alliacea	95.0 ± 0.23 (10.8)	100 ± 0.00 (12.2)	95.0 ± 0.23 (7.47)
U. capitata	40.0 ± 0.00 (24.5)	85.0 ± 0.06* (19.7)	85.0 ± 0.23* (12.8)

* mean germination percentage significantly higher than that of the non-stratified treatment ($P \leq 0.05$)
** mean germination percentage significantly lower than that of the non-stratified treatment ($P \leq 0.05$)

that these species require shallow sowing to germinate. In contrast, seeds of *G. dalenii* germinate better in dark than light, therefore deep sowing in the field would be preferred to achieve a maximum germination rate. *Dianthus basuticus*, *G. dalenii* and *R. lanceolatus* may be stratified for less than 15 days before sowing, as the seeds are sensitive to low temperature (5°C) and germinate during the stratification period. For better percentage germination and low MGT, the seeds of *U. capitata* can be subjected to 30 days' stratification. The MGT of *K. caulescens*, *S. columbaria* and *T. alliacea* seeds can be shortened by 30 days' stratification. The results obtained from this study present preliminary findings. However, further investigation is required to fully explain the factors that control germination and seedlings' survival of these species under field conditions.

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